

Transport of taurine and its regulation by protein kinase C in the JAR human placental choriocarcinoma cell line

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The JAR human placental choriocarcinoma cell line transports taurine, concentrating it over 1000-fold inside the cell. The transport system is energized by a Na⁺ gradient and exhibits an absolute requirement for Cl[−]. Neutral β-amino acids such as β-alanine and hypotaurine effectively compete with the system, whereas neutral α-amino acids such as alanine, leucine and α-aminoisobutyric acid do not. The transport system interacts with γ-aminobutyric acid to an appreciable extent. Kinetic analysis reveals that the taurine transport system in this cell line is of a high-affinity and low-capacity type (apparent dissociation constant 2.3 ± 0.3 μM; maximal velocity 88.5 ± 5.0 pmol/3 min per mg of protein). Pretreatment of the JAR choriocarcinoma cells with phorbol 12-myristate 13-acetate results in the inhibition of the taurine transport system in a dose-dependent manner. The inhibition is blocked by co-treatment of the cells with staurosporine, an inhibitor of protein kinase C. The inactive phorbol ester, 4α-phorbol 12,13-didecanoate, has no effect on the transport system. These data show that the choriocarcinoma cells express a taurine transporter with characteristics similar to those of the taurine transporter described in the normal human placenta, and that the activity of the transporter in these cells is under the regulatory control of protein kinase C.

INTRODUCTION

Taurine (2-aminoethanesulphonic acid), a β-amino acid, has received considerable attention in recent years. Various aspects of taurine, such as its biochemistry and metabolism, its importance in animal nutrition and its biological functions, have been reviewed extensively (Hayes & Sturman, 1981; Chesney, 1985; Wright *et al.*, 1986; Hayes, 1988; Sturman, 1988; Huxtable, 1989). This non-protein amino acid is the most abundant free amino acid in a number of tissues. The nutritional requirements for taurine in man are met partly by dietary sources and partly by biosynthesis from methionine and cysteine. But the biosynthetic capacity exhibits an interesting developmental pattern. It is very low at fetal and neonatal stages, and progressively increases with age to reach the adult levels (Ghisolfi, 1987). In contrast, the tissue levels of taurine decrease with age, being highest in fetal life and lowest in adult life (Ryan & Carver, 1966; Sturman & Gaull, 1975). Thus taurine appears to play an important role in fetal and neonatal development in man, and yet the human fetus and newborn have the least ability to produce the amino acid endogenously. Human milk as well as milk from other sources contain high concentrations of taurine, and intestinal absorption is the primary route by which the newborn obtains this amino acid. Quite understandably however, placental transfer from the mother is the most important mechanism for meeting the nutritional requirements for this amino acid in fetal life.

There is evidence that human placenta transports taurine from mother to fetus by an active process, because the taurine concentration in fetal blood is greater than in maternal blood (Phillips *et al.*, 1978; Yudilevich & Sweiry, 1985). The mechanism by which this active transport occurs has been investigated *in vitro* by using intact human term placentas (Hibbard *et al.*, 1990; Karl & Fisher, 1990) as well as isolated brush-border membrane vesicles (Miyamoto *et al.*, 1988; Kulanthaiyel *et al.*, 1989; Karl & Fisher, 1990). These studies have revealed the presence of a transport system in the brush-border membrane of

the syncytiotrophoblast which is very specific for taurine and other β-amino acids. The transporter is energized by a transmembrane NaCl gradient. Even though the basic characteristics of the transporter appear to have been worked out, many other aspects of the placental handling of taurine remain to be investigated. Placental tissue concentration of taurine is nearly 100–150-fold greater than that in fetal and maternal circulations (Phillips *et al.*, 1978; Yudilevich & Sweiry, 1985). Because this estimate is for the whole placental tissue, which consists of many types of cells, the concentration of taurine in the syncytiotrophoblast is expected to be many times greater than this value. This is undoubtedly the highest placenta-to-plasma ratio of any amino acid. The mechanisms responsible for such a high concentration of taurine in placental tissue are not known. Similarly, there is no information available on the transport process involved in the exit of taurine from the syncytiotrophoblast across the basal membrane. Furthermore, nothing is known on the regulation, if any, of the overall placental transfer of taurine by fetal and/or maternal factors.

The purpose of the present investigation was to determine the suitability of a culture cell line of human placental origin as a model for placental taurine transport studies. The results reported here provide evidence for the presence of a taurine transporter in JAR human choriocarcinoma cells. The transporter expressed in this cell line exhibits characteristics which are similar to those of the taurine transporter described in normal term human placentas. The present study also demonstrates that the activity of the taurine transporter in these cells is under the regulatory control of protein kinase C. This cell line could become a very useful model to understand the various processes involved in the handling of taurine by the normal human placenta.

EXPERIMENTAL

Materials

[2-³H(n)]Taurine (sp. radioactivity 20.1 Ci/mmol) and 3-*O*-[methyl-³H]methyl-D-glucose (sp. radioactivity 79.0 Ci/mmol)

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were purchased from DuPont-New England Nuclear, Boston, MA, U.S.A. Unlabelled amino acids, phorbol esters and staurosporine were obtained from Sigma, St. Louis, MO, U.S.A. RPMI-1640 medium, penicillin/streptomycin, Fungizone (amphotericin B) and fetal-bovine serum were obtained from Flow Laboratories, McLean, VA, U.S.A. All other chemicals were of analytical grade.

Methods

Culture of human placental choriocarcinoma cells (JAR). The JAR cell line was obtained from the American Type Culture Collection, Rockville, MD, U.S.A., and cultured in 75 cm² Corning culture flasks with RPMI-1640 medium supplemented with 10% (v/v) fetal-bovine serum, 50 units of penicillin/streptomycin/ml and 0.125 µg of Fungizone/ml. The cells were maintained at 37 °C in a CO₂ incubator. Confluent cultures were trypsin-treated with phosphate-buffered saline (8.1 mM-Na₂HPO₄, 1.4 mM-KH₂PO₄, 137 mM-NaCl, 2.7 mM-KCl, pH 7.2) containing 0.1% trypsin and 0.25 mM-EDTA, and subcultures were started from the released cells. For the uptake studies, the cells were seeded in 35 mm disposable Falcon Petri dishes at a density of approx. 1.5×10^6 cells/dish and allowed to grow as a monolayer. The cells received 1 ml of fresh culture medium 24 h after the subculture and were used the next day.

Measurement of taurine uptake. The medium was removed from monolayer cultures and replaced with the uptake buffer, which contained 25 mM-Hepes/Tris (pH 7.5), 140 mM-NaCl, 5.4 mM-KCl, 1.8 mM-CaCl₂, 0.8 mM-MgSO₄, 5 mM-D-glucose and radiolabelled taurine. In most cases, the final concentration of [³H]taurine was 20 nM. After incubation at room temperature (22–23 °C) for the desired time, the buffer was removed and the cells were quickly washed four times with the uptake buffer. After washing, 1 ml of 0.3 M-NaOH was added to each dish and left for 1 h, after which time the contents from the dish were transferred to a scintillation vial and the radioactivity of the contents was measured by liquid-scintillation spectrometry.

The procedure for uptake measurement was slightly modified in experiments which were designed to determine the Na⁺-dependence of taurine uptake. Two uptake buffers were used: the regular NaCl-containing buffer and the buffer in which NaCl was replaced by choline chloride. Before the initiation of uptake measurement, the monolayer cultures were incubated with either the NaCl buffer or the choline chloride buffer for 30 min in the absence of radiolabelled taurine. This step was necessary to remove any endogenous Na⁺ when the choline chloride buffer was used. After the preincubation, the corresponding buffer containing radiolabelled taurine was added to the dish and the uptake measurement was initiated.

In the experiments determining the effects of anions on taurine uptake, a modified uptake buffer was used. The uptake buffer contained 25 mM-Hepes/Tris (pH 7.5), 5.4 mM-potassium gluconate, 1.8 mM-calcium gluconate, 0.8 mM-magnesium gluconate, 5 mM-D-glucose and 140 mM of the sodium salt of the respective anion. The medium was removed from the dish, and the uptake buffer containing radiolabelled taurine was added to the monolayer cultures to initiate uptake measurement.

Treatment of the cells with phorbol esters. Stock solutions of phorbol esters and staurosporine were prepared in dimethyl sulphoxide. These solutions were appropriately diluted with the culture medium and used for the treatment of the cells. The final concentration of dimethyl sulphoxide during treatment was 0.02–0.07%, depending on the experiment. The control cells were treated with the respective equal concentration of the solvent in each experiment. After incubation for a desired time, the medium was aspirated and the monolayers were washed once with the uptake buffer before initiation of uptake measurement.

Protein determination. Protein was assayed for each experiment by using duplicate dishes cultured concurrently and under conditions identical with those dishes used for uptake measurements. Deionized water (1 ml) was added to each dish. The dishes were frozen and thawed twice, after which the dish contents were suspended to form a homogenate by using a 1 ml syringe and a 25-gauge needle. Protein concentration of the homogenate was determined by the method of Lowry *et al.* (1951), with BSA as the standard.

Determination of cell number. This was done in those experiments which were designed to measure the intracellular water space. Triplicate dishes cultured concurrently and under conditions identical with those dishes used for protein and uptake measurements were used for determination of the cell number. The cells were released by trypsin treatment and suspended in phosphate-buffered saline, and the cell number was determined with a Coulter counter.

Determination of intracellular water space. This was done from the equilibrium (60 min incubation) distribution of radiolabelled 3-O-methyl-D-glucose. The washing buffer contained 0.5 mM-phlorehizin to prevent the efflux of the sugar during the washing procedure.

Statistics. Each experimental point was determined with duplicate or triplicate dishes, and each experiment was repeated 2–5 times. Statistical analysis of the experimental data was done with the computer statistics package Statgraphics (STSC, Rockville, MD, U.S.A.). The results are expressed as means ± S.E.M.; $P < 0.05$ was considered statistically significant.

RESULTS

Na⁺-dependence and time course of taurine uptake

Fig. 1 describes the time-dependent accumulation of taurine in human placental choriocarcinoma cells. When NaCl was present in the medium, uptake of taurine (0.1 µM) into these cells was very rapid and increased with time. The uptake was linear at least up to 15 min. However, when NaCl in the medium was replaced by choline chloride, uptake of taurine was drastically decreased at all time points. The initial uptake rates measured with a 5 min

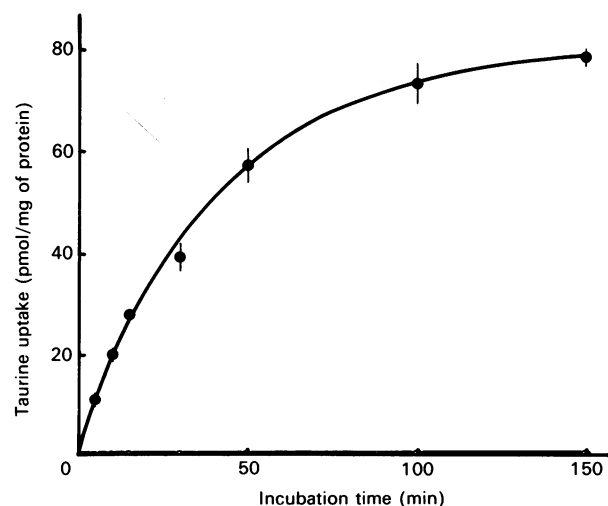


Fig. 1. Time course of taurine uptake in human placental choriocarcinoma cells

Uptake of taurine in choriocarcinoma cells was measured from a medium containing either NaCl (●) or choline chloride (○). Concentration of taurine in the medium at the start of the experiment was 0.1 µM. The data represent means ± S.E.M. ($n = 4$). When not shown, the S.E.M. was within the symbol.

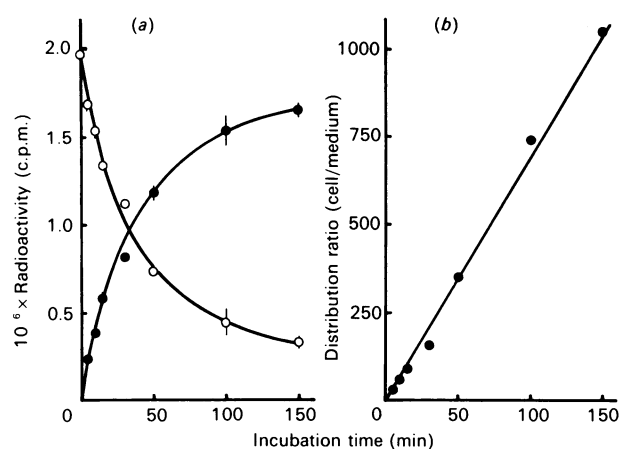


Fig. 2. Cell:medium distribution ratio for taurine in human placental choriocarcinoma cells

Uptake medium (1 ml) containing $0.1 \mu\text{M}$ radiolabelled taurine (2×10^6 c.p.m.) was added to the monolayer cultures at the start of the experiment. At the indicated time intervals, the radioactivity accumulated inside the cells (●) and the radioactivity remaining in the uptake medium (○) were determined (a). From these values, the concentrations of taurine inside the cells and in the medium were calculated and were used to determine the cell:medium distribution ratio (b). The value for intracellular water space which was used in the calculation of intracellular concentration of taurine was $4.4 \mu\text{l/mg}$ of protein. The data represent means \pm S.E.M. ($n = 4$).

Table 1. Anion requirement for the Na^+ -dependent taurine uptake

The values are means \pm S.E.M. ($n = 4$). Uptake of $[^3\text{H}]$ taurine into the monolayers of JAR choriocarcinoma cells was measured with a 3 min incubation. A modified uptake buffer was used in this experiment, and it contained 25 mM-Hepes/Tris (pH 7.5), 5.4 mM-potassium gluconate, 1.8 mM-calcium gluconate, 0.8 mM-magnesium gluconate, 5 mM-D-glucose and 140 mM of the sodium salt of one of the following anions: chloride, iodide, fluoride, nitrate, gluconate and thiocyanate. The concentration of $[^3\text{H}]$ taurine was 20 nM.

Anion	$[^3\text{H}]$ Taurine uptake	
	(pmol/3 min per mg of protein)	(%)
Chloride	0.86 ± 0.13	100
Iodide	0.21 ± 0.01	25
Fluoride	0.18 ± 0.02	21
Nitrate	0.18 ± 0.02	21
Gluconate	0.04 ± 0.01	4
Thiocyanate	0.48 ± 0.02	56

incubation were less than 1 % of those in the presence of NaCl. These data clearly demonstrate the dependence of taurine uptake on Na^+ in JAR choriocarcinoma cells. The presence of Cl^- alone was not enough to support the uptake.

Cell:medium distribution ratio

During the initial studies involving the time course of taurine uptake, it became evident that the human placental choriocarcinoma cells possess an exceptional ability to accumulate taurine against a concentration gradient. Fig. 2(a), in which a comparison is made between the time-dependent accumulation of radioactivity in the form of $[^3\text{H}]$ taurine and the corresponding time-dependent decrease in the radioactivity in the medium, clearly illustrates the capacity of these cells to accumulate taurine.

At the start of the experiment, 1 ml of the NaCl medium containing 2×10^6 c.p.m. ($0.1 \mu\text{M}$ - $[^3\text{H}]$ taurine) was added to the monolayer cultures. Uptake was allowed to proceed for the indicated time intervals, at the end of which the amounts of radioactivity inside the cells and in the medium were determined. The cells were able to take up 50 % of taurine presented to them in the medium within 30–40 min of incubation. The ability of the cells to accumulate taurine continued with time, and at 150 min the cells had accumulated approx. 85 % of the taurine presented to them.

To quantify the ability of human choriocarcinoma cells to transport taurine against a concentration gradient, we first determined the intracellular water space. This was done from the equilibrium distribution of radiolabelled 3-O-methyl-D-glucose, a non-metabolizable sugar. This sugar is transported into these cells by facilitated diffusion via the glucose transporter and it is not concentrated against a gradient. Therefore the intracellular water space can be calculated by determining the distribution of this sugar inside the cells and in the medium at equilibrium. Initial experiments showed that the uptake of 3-O-methyl-D-glucose into choriocarcinoma cells reached equilibrium within 60 min of incubation. Therefore a 60 min incubation was chosen for the experiment. By using these equilibrium conditions, the intracellular water space was determined to be $4.4 \pm 0.4 \mu\text{l/mg}$ of protein or $1.07 \pm 0.08 \mu\text{l}/10^6$ cells. With this value for the intracellular water space, the intracellular concentration of taurine was determined. Because the concentration of taurine in the medium at the end of each incubation period is known (Fig. 2a), the cell:medium distribution ratio could be determined for each time point. The results are given in Fig. 2(b). The ability of these cells to accumulate taurine against a concentration gradient was evident even at the shortest time of incubation used in the experiment (the cell:medium ratio was approx. 30 at 5 min). The ratio increased almost linearly with time, and the value was greater than 1000 at 150 min incubation. These results demonstrate that human choriocarcinoma cells possess a tremendous ability to concentrate taurine by transporting the amino acid from the medium against a concentration gradient.

Anion specificity

Fig. 1 described the dependence of taurine uptake on the presence of Na^+ in human choriocarcinoma cells. We also investigated whether taurine transport in these cells exhibits any specific requirement for anions. In this experiment, the initial rates of taurine uptake were determined by using a 3 min incubation in the presence of the sodium salt of different anions. The results given in Table 1 demonstrate that the uptake rates were maximal in the presence of Cl^- . The anions I^- , F^- , NO_3^- and gluconate supported the uptake only minimally. Appreciable uptake rates were however observed in the presence of SCN^- . Therefore, there is a specific requirement for Na^+ as well as Cl^- for optimal transport of taurine in these cells.

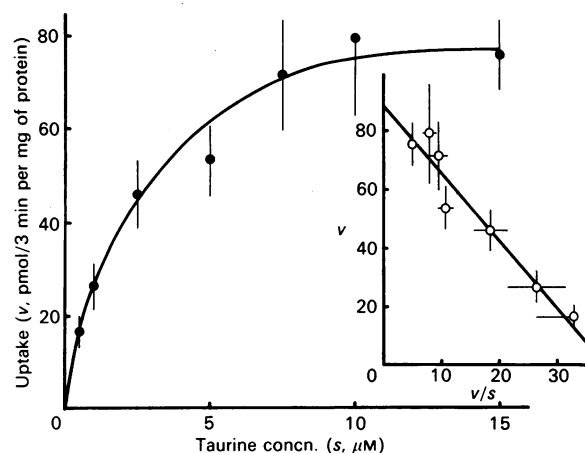
Substrate specificity

The substrate specificity of the carrier system responsible for taurine uptake in human choriocarcinoma cells was investigated by studying the effects of a variety of unlabelled amino acids on the uptake of radiolabelled taurine. There are different group-specific amino acid transport systems which serve for the uptake of neutral amino acids in animal cells (Christensen, 1985). There are at least three systems for neutral α -amino acids (A, ASC and I), but there is only a single system which accepts neutral β -amino acids as substrates (system β). To determine which of these transport systems is responsible for the uptake of taurine in human placental choriocarcinoma cells, we examined the effects of the following unlabelled amino acids on the uptake of

Table 2. Inhibition of [³H]taurine uptake by unlabelled amino acids

The values are means \pm S.E.M. ($n = 4$). Uptake of [³H]taurine into the monolayers of JAR choriocarcinoma cells was measured with a 3 min incubation. The concentration of [³H]taurine was 20 nM and that of unlabelled amino acids was 100 μ M.

Amino acid (100 μ M)	[³ H]Taurine uptake	
	(pmol/3 min per mg of protein)	(%)
Control	0.83 ± 0.06	100
Taurine	0.02 ± 0.01	2
Hypotaurine	0.02 ± 0.01	2
β -Alanine	0.06 ± 0.01	7
γ -Aminobutyric acid	0.46 ± 0.12	56
Alanine	0.74 ± 0.12	90
Leucine	0.93 ± 0.07	112
α -Aminoisobutyric acid	0.76 ± 0.10	92

**Fig. 3. Kinetic analysis of taurine uptake in human choriocarcinoma cells**

Initial uptake rates of taurine were determined by using a 3 min incubation. The range of taurine concentration employed in the experiment was 0.5–15 μ M. Inset: Eadie-Hofstee plot. The data represent means \pm S.E.M. ($n = 4$).

[³H]taurine: taurine, β -alanine and hypotaurine (system- β -specific amino acids), alanine (system-ASC-specific amino acid), leucine (system-I-specific amino acid), α -aminoisobutyric acid (system-A-specific amino acid) (Table 2). At a concentration of 100 μ M, the amino acids specific for systems A, ASC and I failed to inhibit the uptake of 20 nM-[³H]taurine. On the contrary, the system- β -specific amino acids almost completely inhibited [³H]taurine uptake under similar conditions. γ -Aminobutyric acid, however, showed an appreciable competition with taurine for the uptake system. Therefore the transport system responsible for taurine uptake in human choriocarcinoma cells is specific for neutral β -amino acids and does not accept neutral α -amino acids as substrates.

Kinetics of taurine uptake

The kinetics of taurine uptake in human placental choriocarcinoma cells were analysed by studying the relationship between the initial uptake rates (3 min incubation) and taurine

Table 3. Effect of treatment time on the inhibition of taurine uptake induced by phorbol 12-myristate 13-acetate

The values are means \pm S.E.M. ($n = 4$). Monolayer cultures were treated with dimethyl sulphoxide or phorbol 12-myristate 13-acetate dissolved in dimethyl sulphoxide for different periods. During this treatment, the concentration of dimethyl sulphoxide was 0.02% and that of the phorbol ester was 1 μ M. After the treatment, the media were aspirated and the cell layers washed once with the uptake buffer before initiation of [³H]taurine uptake. The concentration of [³H]taurine was 20 nM and the incubation period was 3 min. For each time period for the treatment with the phorbol ester, the cells treated with dimethyl sulphoxide for the corresponding time period served as the control.

Treatment time (min)	Inhibition of [³ H]taurine uptake (%)
15	9 ± 6 ($P < 0.01$)
30	33 ± 1 ($P < 0.001$)
60	36 ± 4 ($P < 0.001$)
120	24 ± 1 ($P < 0.001$)

concentration. The uptake rates were determined over a taurine concentration range of 0.5–15 μ M. At each concentration, the uptake medium contained 0.04 μ M radiolabelled taurine and the remainder as unlabelled taurine. Non-carrier-mediated uptake was determined by measuring the uptake of radiolabel in the presence of 100 μ M unlabelled taurine. When compared with the values for radiolabel uptake in the presence of 0.5 μ M and 15 μ M unlabelled taurine, the radiolabel uptake in the presence of 100 μ M unlabelled taurine was 3.9 ± 0.4 and $18.8 \pm 0.6\%$ respectively. This value was subtracted from total uptake to calculate carrier-mediated uptake. The relationship between the initial rates of carrier-mediated uptake and taurine concentration is illustrated in Fig. 3. The uptake rate was clearly a hyperbolic function of taurine concentration, suggesting participation of a saturable transport system mediating taurine uptake in these cells. When these data were transformed into a linear format by the Eadie-Hofstee method (initial uptake rate/taurine concentration versus initial uptake rate), a linear plot ($r = -0.97$) was obtained (Fig. 3, inset). These data suggested the involvement of a single system. The linear transformation was used to determine the kinetic parameters K_t (the apparent dissociation constant) and V_{\max} (the maximal velocity). The K_t was 2.3 ± 0.3 μ M and the V_{\max} was 88.5 ± 5.0 pmol/3 min per mg of protein.

Effects of phorbol esters on the activity of the taurine transporter

We investigated the possible involvement of protein kinase C in the regulation of the taurine transporter by studying the influence of phorbol 12-myristate 13-acetate on taurine uptake in the JAR choriocarcinoma cells. The monolayer cultures were treated with the phorbol ester at a concentration of 1 μ M for 15–120 min before initiation of uptake measurement. Since the stock solutions of the ester were made in dimethyl sulphoxide, the control monolayers were treated in a similar manner with an equal concentration of the solvent alone. The data given in Table 3 show that treatment of the cells with the phorbol ester resulted in inhibition of taurine uptake, and the magnitude of inhibition was dependent on the duration of treatment of the cells with the ester. A small but significant ($P < 0.01$) inhibition was observed even at a treatment time as short as 15 min. Maximal inhibition (approx. 35%) occurred between 30 and 60 min. Increasing the treatment time beyond 60 min did not increase the inhibition,

Table 4. Concentration-dependent inhibition of taurine uptake by phorbol 12-myristate 13-acetate (PMA)

The values are means \pm S.E.M. ($n = 6$). The monolayer cultures were treated with the indicated concentrations of PMA for 60 min before initiation of uptake measurement. Uptake of 20 nM-[3 H]taurine was measured with a 3 min incubation. Control uptake was 0.85 ± 0.04 pmol/3 min per mg of protein (100 %).

Concn. of PMA (nM)	Inhibition of [3 H]taurine uptake (%)
0.1	8 ± 2 ($P < 0.05$)
1	10 ± 1 ($P < 0.002$)
10	16 ± 1 ($P < 0.001$)
100	22 ± 3 ($P < 0.001$)
1000	30 ± 2 ($P < 0.001$)

Table 5. Influence of staurosporine on the phorbol-ester-induced inhibition of taurine uptake

The values are means \pm S.E.M. ($n = 10$). Monolayer cultures were treated with dimethyl sulphoxide (control), phorbol 12-myristate 13-acetate (PMA), 4 α -phorbol 12,13-didecanoate (PDD), staurosporine or staurosporine plus PMA for 60 min before the initiation of uptake measurement. The final concentrations of the reagents were: dimethyl sulphoxide, 0.07 %; PMA, 1 μ M; PDD, 1 μ M; staurosporine, 0.5 μ M. Uptake of 20 nM-[3 H]taurine was measured with a 3 min incubation. Control uptake was 0.80 ± 0.01 pmol/3 min per mg of protein (100 %). NS, not significant. ^a, compared with the control; ^b, compared with the treatment with staurosporine alone.

Compound	[3 H]Taurine uptake (%)
Control	100 ± 1
PMA	69 ± 2 ($P < 0.001$) ^a
PDD	96 ± 4 (NS) ^a
Staurosporine	120 ± 3 ($P < 0.001$) ^a
Staurosporine + PMA	124 ± 4 (NS) ^b

but rather decreased it. An incubation period of 60 min was therefore used in subsequent experiments to characterize the phorbol-ester-induced inhibition of the taurine transporter.

Table 4 describes the dose/response of the inhibition. At a concentration of 1 μ M, the phorbol ester caused 30 ± 2 % inhibition. The inhibition was significant (16 ± 1 %; $P < 0.001$) even at a concentration as low as 10 nM.

Involvement of protein kinase C in the phorbol-ester-induced inhibition of the taurine transporter

Phorbol 12-myristate 13-acetate is a tumour promoter which is capable of activating protein kinase C (Ca^{2+} - and phospholipid-dependent protein kinase) (Castagna *et al.*, 1982; Nishizuka, 1984). In order to determine if protein kinase C is involved in the phorbol-ester-induced inhibition of the taurine transporter in the JAR choriocarcinoma cell line, we studied the effect of 4 α -phorbol 12,13-didecanoate, an analogue of phorbol 12-myristate 13-acetate, which is unable to activate protein kinase C (Kraft *et al.*, 1982). The results given in Table 5 show that taurine uptake was inhibited by 31 % ($P < 0.001$) when the cells were treated with 1 μ M-phorbol 12-myristate 13-acetate. On the contrary, the inactive analogue failed to show any effect on the taurine transporter under similar experimental conditions.

We also determined the influence of staurosporine, a potent

inhibitor of protein kinase C (Tamaoki *et al.*, 1986), on taurine uptake in the absence and presence of phorbol 12-myristate 13-acetate (Table 5). Treatment of the cells with staurosporine (0.5 μ M) alone caused a small but significant (20 %; $P < 0.001$) stimulation of taurine uptake. When the cells were treated with phorbol 12-myristate 13-acetate in the presence of staurosporine, taurine uptake in these cells was not significantly different from the uptake in cells treated with staurosporine alone ($P = 0.45$). These results show that the phorbol-ester-induced inhibition of taurine uptake is completely blocked by staurosporine.

DISCUSSION

In this investigation, we have demonstrated that the JAR human placental choriocarcinoma cell line expresses a high-affinity taurine transporter with characteristics similar to those of the taurine transporter expressed in the normal human term placenta. In addition, this study provides the first evidence for the regulation of the taurine transporter by protein kinase C.

The significance of the results presented here is many-fold. The presence of a mechanism for active uptake of taurine has been demonstrated in a variety of tissues, but most of these investigations have been performed in laboratory animals. The general properties of the transport system are similar, if not identical, in all these tissues. However, the kinetic parameters, the kinetically distinguishable number of transport systems, and relative affinities for various substrates vary significantly among the tissues. Moreover, these characteristics also exhibit species differences. The taurine transport system has been studied in very few human tissues (Ahtee *et al.*, 1974; Schmidt & Berson, 1980; Miyamoto *et al.*, 1988; Kulanthaivel *et al.*, 1989; Karl & Fisher, 1990; Hibbard *et al.*, 1990). There are two reports on the characteristics of taurine uptake in cultured cells of human origin, fibroblasts (Thompson, 1988) and lymphoblastoid cells (Tallan *et al.*, 1983). Thus knowledge of the taurine transport system in tissues and cells of human origin is very limited. The present demonstration that human placental choriocarcinoma cells possess the taurine transport system makes this cell line a convenient model to investigate the biochemical and regulatory aspects of the transport system.

Another area of investigation in which the JAR choriocarcinoma cell line can become potentially useful is the cellular function of taurine. The proposed biochemical functions of taurine in mammalian cells for which there is supporting experimental evidence include osmoregulation, membrane protection, antioxidant and interaction with cellular signalling pathways such as Ca^{2+} and cyclic nucleotides. The unique way in which taurine is handled by many tissues suggests that this amino acid may have other, more fundamental, biochemical functions. Many types of cells have an exceptional ability to accumulate taurine against a concentration gradient. The cell-to-extracellular-medium ratio has been reported to be as high as 400 for retina (Pasantes-Morales, 1986), 2000 for Ehrlich ascites-tumour cells (Christensen *et al.*, 1954) and 7000 for HeLa cells (Piez & Eagle, 1958). Normal human placental tissue also accumulates taurine to a great extent, and a tissue-to-plasma ratio as high as 150 has been reported (Phillips *et al.*, 1978). The exceptional ability of the normal placenta to concentrate taurine raises the possibility that the function of the placental tissue may be modulated by taurine. All of the biochemical effects of taurine that have been demonstrated in other tissues have the potential to contribute to the regulation of the placental function, if it can be shown that taurine has similar effects in the placental tissue. But, surprisingly, there is no information available on the biological role of taurine in the human placenta. The JAR human choriocarcinoma cell line, which has been shown in the

present study to have the mechanisms to concentrate taurine as the normal placenta does, can be used as an experimental system to investigate the modulation of placental function by taurine.

In addition to supplying taurine to the syncytiotrophoblast for cellular functions, the transport system is also obligatory for trans-placental transfer of taurine from mother to fetus. This function is of paramount importance for the well-being of the fetus. There is overwhelming evidence for a special role of taurine in development (Sturman, 1988). The observations that fetal tissues, especially brain, contain higher levels of taurine than mature tissues argue for such a role. Studies in laboratory animals have demonstrated that induction of taurine deficiency has profound effects on reproduction and pregnancy outcome in females. The aborted fetuses, stillborn pups and surviving newborns of taurine-deficient mothers have developmental abnormalities, indicating an important function for taurine in cellular proliferation and differentiation. Therefore, placental transfer of taurine assumes great importance in the supply of taurine to the fetus, and thus is crucial to optimal growth and development of the fetus. There are a few studies on the placental handling of taurine in laboratory animals (Lemmons *et al.*, 1976; Sturman *et al.*, 1977; Eaton & Yudilevich, 1981; Stegink *et al.*, 1981), but these studies have produced contradictory results, suggesting significant species variations. Investigations on the transport of taurine in human placenta are limited, and consequently there is a dearth of knowledge regarding the molecular mechanisms of the human placental taurine transport system. The human choriocarcinoma cell line can become potentially useful in advancing our understanding of this transport system.

Our observations that the activity of the taurine transporter in the JAR choriocarcinoma cells is regulated by protein kinase C raise the possibility that the taurine transporter in the human placenta may also be subject to regulation by protein kinase C. Relevant to this point are reports (Moore *et al.*, 1986; Tertrin-Clary *et al.*, 1990) which describe the existence of protein kinase C, as well as several proteins which are phosphorylated by protein kinase C, in the human placenta. It is also known that the placenta possesses receptors for various hormones and growth factors (e.g. gonadotropin-releasing hormone, epidermal growth factor) which utilize signalling mechanisms involving protein kinase C. Therefore, it is likely that these agents modulate the activity of the placental taurine transporter under physiological conditions.

This work was in part supported by the National Institutes of Health Grant HD 24451. We thank Marcia D. Lewis for excellent secretarial assistance.

REFERENCES

- Ahtee, L., Boullin, D. J. & Paasonen, M. K. (1974) *Br. J. Pharmacol.* **52**, 245–251
- Castagna, M., Takai, Y., Kaibuchi, K., Sano, K., Kikkawa, U. & Nishizuka, Y. (1982) *J. Biol. Chem.* **257**, 7847–7851
- Chesney, R. W. (1985) *Adv. Pediatr.* **32**, 1–42
- Christensen, H. N. (1985) *J. Membr. Biol.* **84**, 97–103
- Christensen, H. N., Hess, B. & Riggs, T. R. (1954) *Cancer Res.* **14**, 124–127
- Eaton, B. M. & Yudilevich, D. L. (1981) *Am. J. Physiol.* **241**, C106–C112
- Ghisolfi, J. (1987) *Biol. Neonate* **52**, Suppl. 1, 78–86
- Hayes, K. C. (1988) *Nutr. Res. Rev.* **1**, 99–113
- Hayes, K. C. & Sturman, J. A. (1981) *Annu. Rev. Nutr.* **1**, 401–425
- Hibbard, J. U., Pridjian, G., Whittington, P. F. & Moawad, A. H. (1990) *Pediatr. Res.* **27**, 80–84
- Huxtable, R. J. (1989) *Prog. Neurobiol.* **32**, 471–533
- Karl, P. I. & Fisher, S. E. (1990) *Am. J. Physiol.* **258**, C443–C451
- Kraft, A. S., Anderson, W. B., Cooper, H. L. & Sando, J. J. (1982) *J. Biol. Chem.* **257**, 13193–13196
- Kulanthaivel, P., Leibach, F. H., Mahesh, V. B. & Ganapathy, V. (1989) *Biochim. Biophys. Acta* **985**, 139–146
- Lemmons, J. A., Adcock, E. W., Jones, M. D., Jr., Naughton, M. A., Meschia, G. & Battaglia, F. C. (1976) *J. Clin. Invest.* **58**, 1428–1434
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Miyamoto, Y., Balkovetz, D. F., Leibach, F. H., Mahesh, V. B. & Ganapathy, V. (1988) *FEBS Lett.* **231**, 263–267
- Moore, J. J., Moore, R. & Cardaman, R. C. (1986) *Proc. Soc. Exp. Biol. Med.* **182**, 364–371
- Nishizuka, Y. (1984) *Nature (London)* **308**, 693–698
- Pasantes-Morales, H. (1986) *Prog. Retinal Res.* **5**, 207–230
- Phillips, A. F., Holzman, I. R., Teng, C. & Battaglia, F. C. (1978) *Am. J. Obstet. Gynecol.* **131**, 881–887
- Piez, K. A. & Eagle, H. (1958) *J. Biol. Chem.* **231**, 533–545
- Ryan, W. L. & Carver, M. J. (1966) *Nature (London)* **212**, 292–293
- Schmidt, S. Y. & Berson, E. J. (1980) *Invest. Ophthalmol. Visual Sci.* **19**, 1274–1280
- Stegink, L. D., Reynolds, W. A., Pitkin, R. M. & Cruikshank, D. P. (1981) *Am. J. Clin. Nutr.* **34**, 2685–2692
- Sturman, J. A. (1988) *J. Nutr.* **118**, 1169–1176
- Sturman, J. A. & Gaull, G. E. (1975) *J. Neurochem.* **25**, 831–835
- Sturman, J. A., Ressin, D. K. & Gaull, G. E. (1977) *J. Neurochem.* **28**, 31–39
- Tallan, H. H., Jacobson, E., Wright, C. E., Schneidman, K. & Gaull, G. E. (1983) *Life Sci.* **33**, 1853–1860
- Tamaoki, T., Nomoto, H., Takahashi, I., Kato, Y., Morimoto, M. & Tomita, F. (1986) *Biochem. Biophys. Res. Commun.* **135**, 397–402
- Tertrin-Clary, C., Chenut, M. C. & DeLa Llosa, P. (1990) *Placenta* **11**, 27–33
- Thompson, G. N. (1988) *Biochem. Cell Biol.* **66**, 702–706
- Wright, C. E., Tallan, H. H., Lin, Y. Y. & Gaull, G. E. (1986) *Annu. Rev. Biochem.* **55**, 427–453
- Yudilevich, D. L. & Sweiry, J. H. (1985) *Biochim. Biophys. Acta* **822**, 169–201